

518,949

Rec'd PCT/PTO 17 DEC 2004

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
29 January 2004 (29.01.2004)

PCT

(10) International Publication Number  
**WO 2004/009827 A2**

- (51) International Patent Classification<sup>7</sup>: **C12P 7/00**
- (21) International Application Number: PCT/EP2003/006552
- (22) International Filing Date: 20 June 2003 (20.06.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
02254262.5 19 June 2002 (19.06.2002) EP  
02258713.3 18 December 2002 (18.12.2002) EP  
03251169.3 26 February 2003 (26.02.2003) EP
- (71) Applicant (for all designated States except US): **DSM IP ASSETS B.V.** [NL/NL]; Het Overloon 1, NL-6411 Te Heerlen (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **STREEKSTRA, Hugo** [NL/NL]; Weteringstraat 28-1, NL-1017 SP Amsterdam (NL). **BROCKEN, Petrus, Joseph, Maria** [NL/NL]; Zwaluw 2, NL-2678 XC de Lier (NL).
- (74) Agents: **WRIGHT, Simon, Mark et al.**; J.A. Kemp & Co, 14 South Square, Gray's Inn, London WC1R 5JJ (GB).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- without international search report and to be republished upon receipt of that report
  - with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PREPARATION OF MICROBIAL OIL

(57) **Abstract:** The present invention provides a process for the production of a microbial oil comprising culturing a micro-organism in a two stage fermentation process where, in a last stage that precedes the end of fermentation, the carbon source is: consumed by the micro-organisms at a rate greater than it is added to the medium; added at a rate  $\leq 0.30$  M carbon/kg medium; or is rate limiting on the growth of the micro-organism. The micro-organisms thus have the carbon source restricted so that they preferentially metabolise fats or lipids other than arachidonic acid (ARA), so increasing the proportion of ARA in the cells. A microbial oil is then recovered from the micro-organism, using hexane as a solvent, that has at least 50% ARA and at least 90% triglycerides.

WO 2004/009827 A2